

# Immunofluorescence of *Drosophila* tissues

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 An abbreviated version of this protocol was published in eLIFE in Jun 2021

RAL GTPases mediate EGFR-driven intestinal stem cell proliferation and tumourigenesis

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## Detailed protocol

### Immunofluorescence of *Drosophila* tissues

Tissues were dissected and fixed in 4% para-formaldehyde (Polysciences, Inc.) at room temperature for a minimum of 30 min. After fixation, tissues were washed 3 times in PBS + 0.2% TritonX-100 (PBST) for 20 min, followed by overnight incubation at 4°C with primary antibodies in PBST + 0.5% Bovine Serum Albumin (BSA) (PBT). Samples were then washed in PBST 3 x 20 minutes and incubated with secondary antibodies in PBT for 3h at room temperature followed by washing and mounting.

Midguts stained for pERK rabbit anti-p-Erk 1:100-Cell Signaling, #9101), included a methanol fixation step between PFA fixation and PBST washing steps of the standard protocol. Following PFA fixation methanol was added dropwise to the solution, with the tissues in it until the volume of the liquid is at least double. Tissues were transferred into 100% methanol for minimum 1 minute. PBS was added to the methanol dropwise to rehydrate the tissues after which the samples were subjected to the standard staining protocol.

All samples were mounted onto glass slides (VWR) with 13mm x 0.12mm spacers (Electron Microscopy Science) and Vectashield antifade mounting medium containing DAPI (Vector Laboratories, Inc). Confocal images were obtained on a Zeiss LSM 780 and processed in the Zeiss ZEN software.

**How to cite:** (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Cordero, J. (2023). Immunofluorescence of *Drosophila* tissues. Bio-protocol Preprint. [bio-protocol.org/prep2102](https://bio-protocol.org/prep2102).
2. Nászai, M., Bellec, K., Yu, Y., Román-Fernández, A., Sandilands, E., Johansson, J., Campbell, A. D., Norman, J. C., Sansom, O. J., Bryant, D. M. and Cordero, J. B. (2021). RAL GTPases mediate EGFR-driven intestinal stem cell proliferation and tumourigenesis. eLIFE. DOI: [10.7554/eLife.63807](https://doi.org/10.7554/eLife.63807)

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